



Macrophage-derived osteopontin is fragmented by MMP-9 to hinder angiogenesis in the post-myocardial infarction left ventricle

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sound to assess aortic stenosis and PET/computed tomography (CT) with ^{18}F -NaF to assess calcification severity. Thereafter, 8 animals were subsequently treated with local delivery of a mixture containing 500 $\mu\text{g/l}$ zoledronate that was delivered on the cusps of the aortic valve, by a dedicated balloon catheter. A placebo mixture was administered on the rest 8 animals (control group). At 28 days all animals underwent a follow-up cardiac imaging with PET-CT ^{18}F -NaF. The progression of calcification was assessed by calculating the difference in SUVmax, SUVmean, TBRmax and TBR mean at baseline and at follow up both for AV and ascending aorta (AA). After the second PET/CT examination all animals were sacrificed and all aortic valves were collected and analyzed by histology.

Results: At baseline, all animals developed aortic valve stenosis with severe calcification. No differences regarding AVA were recorded between both groups. (21.37 ± 1.76 vs 21.98 ± 3.12 , $p=0.53$). In all animals the local delivery of zoledronate and placebo mixtures was successful and uncomplicated. A total of 48 cusps were histologically examined. The cusps treated with zoledronate had significantly lower expression of calcium content compared to the cusps of the placebo group (16.40 ± 0.90 vs $24.88 \pm 1.90\%$ of the area, $p<0.0001$), whereas the ascending aortas of both groups showed similar expression of calcium content (23.58 ± 4.43 vs $23.12 \pm 5.05\%$ of the area, $p=0.78$). Regarding PET/CT analysis, in the zoledronate group, TBRmean and TBRmax at the level of AA showed a significant increase of calcification during follow up (1.31 ± 0.11 versus 1.63 ± 1.84 , $p=0.001$ and 1.42 ± 0.11 versus 1.64 ± 0.20 , $p=0.001$). In the same group TBRmean and TBRmax at the level of the AV did not show any significant change in calcification during the same period (1.20 ± 0.12 versus 1.17 ± 0.78 , $p=0.29$ and 1.30 ± 0.33 versus 1.40 ± 0.67 , $p=0.08$). Interestingly TBRmean showed a regression of calcification at the level of AV compared to AA (-0.34 ± 0.07 versus 0.30 ± 0.11 , $p=0.01$).

Conclusion: Inhibition of aortic valve calcification by local catheter-based delivery of zoledronate is feasible without evident short-term complications. The potential clinical implications should be confirmed in human studies

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Macrophage-derived osteopontin is fragmented by MMP-9 to hinder angiogenesis in the post-myocardial infarction left ventricle

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Background/Introduction: Extracellular matrix (ECM) turnover is a key event during remodeling of the left ventricle (LV) following myocardial infarction (MI). Turnover includes ECM degradation of existing ECM to remove necrotic myocytes and synthesis to produce new ECM to form the infarct scar. Matrix metalloproteinases (MMPs) are elevated post-MI, and MMP-9 has a strong link to post-MI LV dysfunction. The ECM protein osteopontin (OPN) increases post-MI, and we previously identified by mass spectrometry a novel MMP-9 cleavage site of OPN between amino acids 151 and 152. In vitro, peptides both upstream and downstream of the cleavage site increased cardiac fibroblast migration without affecting proliferation.

Purpose: The aim was to determine the biological function of the MMP-9 generated OPN fragments in vivo post-MI, using full length and cleavage-site specific OPN antibodies.

Methods: C57/BL6J wild type (WT) and MMP-9 null mice (3–6 months old) were used for coronary artery ligation and examined at days 0, 1, 3, 5, and 7 post-MI. All animal procedures were approved by the Institutional Animal Care and Use Committee at a University Medical Center in accordance with the Guide for the Care and Use of Laboratory Animals. Immunoblotting and immunohistochemistry were used to quantify full-length and OPN fragments. In vitro angiogenesis assay was performed using HUVECs to compare spanning OPN fragment peptide to fragment peptides upstream and downstream of the cleavage site.

Results: In vivo, both full length OPN and the cleaved OPN fragment increased in the LV infarct in WT from days 1 to 5, with a peak elevation at day 5 post-MI. Compared to WT, post-MI MMP-9 null LV showed a surprising increase in cleavage product, indicating that MMP-9 may further degrade OPN with prolonged exposure. This was confirmed by in vitro cleavage assay. By immunohistochemistry, we identified myocytes as the main cellular source of OPN in the absence of MI. Post-MI, macrophages were robustly positive for both full length OPN and the cleaved fragment. An angiogenesis assay was performed to further elucidate the biological function of MMP-9 generated OPN fragments. While total tubular formation was not altered, the OPN spanning peptide increased total branching and segment length, effects that were abolished when peptides upstream or downstream of the cleavage site were used.

Conclusions: Our results demonstrated that in vivo post-MI, MMP-9 increased OPN, which was proteolytically processed as a result of macrophage infiltration, and in vitro cleavage peptides impaired angiogenesis quality.

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Mitochondrial calpains mediate SIRT3-dependent cardiac dysfunction in LPS-induced endotoxemia

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Sepsis may result in myocardial dysfunction, likely related to concomitant mitochondrial dysfunction in the heart. Sirtuin 3 (SIRT3) is a mitochondrial NAD⁺-dependent deacetylase, lack of which impairs mitochondrial ATP synthesis by decreased deacetylation of proteins of fatty acid oxidation, the TCA cycle and electron transport chain. Since sepsis is characterized by hyperactivation of the NAD⁺-dependent DNA repair enzyme poly(ADP-ribose)polymerase-1 (PARP1), we hypothesized that myocardial NAD⁺ depletion due to myocardial PARP1 activation may impair SIRT3 activity and thereby contribute to mitochondrial and contractile dysfunction in sepsis. In isolated working hearts, 6 hours of LPS treatment resulted in a decrease in cardiac power (-22%), palmitate oxidation (-33%) and cardiac efficiency (-34%), accompanied by a 57% decrease of the myocardial NAD⁺/NADH ratio compared to non-treated mice (all $p<0.05$). PARP1 deletion lead to a complete recovery of cardiac function and NAD⁺ levels in LPS-treated mice, which was completely blunted in LPS-treated PARP1^{-/-}/SIRT3^{-/-} double knockout mice. Mitochondrial rates of ATP synthesis were decreased in LPS-treated WT mice (-23%) and LPS-treated SIRT3^{-/-} mice (-21%; all $p<0.05$). ATP synthesis was completely normalized in LPS-treated PARP1^{-/-} mice, but not in LPS-treated PARP1^{-/-}/SIRT3^{-/-} mice. Since mitochondrial calpains cause mitochondrial dysfunction in sepsis by cleavage of ATP synthase, mice were treated with the calpain inhibitor MDL-28170. MDL treatment resulted in a 81% improvement of cardiac function in LPS-treated WT mice ($p<0.05$), but not in LPS-treated SIRT3^{-/-} mice. Thus, LPS-induced endotoxemia causes cardiac dysfunction by PARP1-mediated NAD⁺ depletion and subsequent impairment in SIRT3 activity, which may increase activation of mitochondrial calpains.

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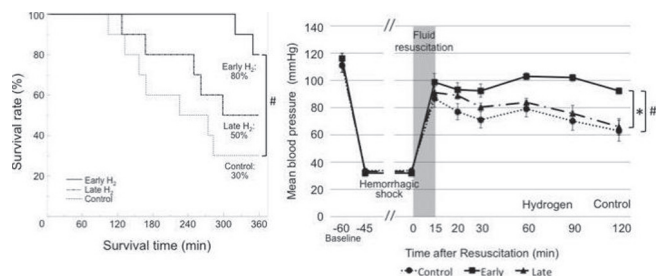
Hydrogen gas inhalation improves survival in rats with lethal haemorrhagic shock

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Background: Haemorrhage is a major perioperative complication and is the primary cause of death following trauma among both civilians and military personnel. Whether patients can endure the state of shock due to haemorrhage until definitive treatment is applied, affects their probability of survival. Hydrogen (H₂) gas has potential as an antioxidant. We investigated whether H₂ gas inhalation affects the tolerance to haemorrhagic shock and improves survival.

Methods: Thirty male Sprague-Dawley rats were anesthetised and subjected to haemorrhagic shock by withdrawing blood until the mean arterial blood pressure reached 30–35 mmHg. After 60 minutes of shock, rats were resuscitated with normal saline measuring four times the volume of shed blood over 15 minutes. The rats were assigned to one of three groups: the early H₂ group (the gas mixture [1.3% H₂, 26% O₂, and 72.7% N₂] was inhaled since the initiation of haemorrhagic shock, N=10), late H₂ group (the gas mixture was inhaled since the initiation of fluid resuscitation, N=10), and control group (a control gas mixture [26% O₂ and 74% N₂] was inhaled since the initiation of haemorrhagic shock, N=10). Inhalation of the specified gas mixture continued for 2 hours after fluid resuscitation. The survival rate at 6 hours after fluid resuscitation and haemodynamic parameters were evaluated.

Results: The survival rate was 30% in the control group, 80% in the early H₂ group, and 50% in the late H₂ group ($P=0.036$). The volume of blood removed to induce shock tended to be the largest in the early H₂ group (control vs. early H₂ vs. late H₂ groups: 2.42 ± 0.12 vs. 2.76 ± 0.11 vs. 2.44 ± 0.11 mL/100 g; $P=0.082$). Fluid resuscitation almost completely restored the blood pressure in the early H₂ group, whereas it failed to fully restore the blood pressure in the late H₂ and control



$P<0.05$ compared with the control group, * $P<0.05$ compared with the late H₂ group
Survival curve and changes in BP